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Exclusion of Allelism of Noonan Syndrome and Neurofibromatosis-Type 1 in a Large Family With Noonan Syndrome-Neurofibromatosis Association

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A large four-generation family with Noonan syndrome (NS) and neurofibromatosis-type 1 (NF1) was studied for clinical association between the two diseases and for linkage analysis with polymorphic DNA markers of the NF1 region in 17q11.2. Nonrandom segregation between NS and NF1 phenotypes was observed. Neurofibromatosis was tightly linked to NF1 markers, whereas Noonan syndrome was found not be allelic to NF1. These results suggest that two mutations at two independent but closely linked loci are the cause of neurofibromatosis-Noonan syndrome (NF-NS) association in this family. © 1996 Wiley-Liss, Inc.

KEY WORDS: Noonan syndrome, neurofibromatosis-type 1, clinical association, linkage exclusion

INTRODUCTION

Noonan syndrome (NS) is a common disorder in humans (~1/2,000 live births) transmitted as an autosomal dominant trait [Noonan and Ehmke, 1963; Mendez and Opitz, 1985]. This syndrome is characterized by small stature, facial anomalies, and impairment of connective tissue involving skeleton and integument. Visceral abnormalities are not rare. Among cardiovascular defects, valvular pulmonary stenosis is considered to be the most specific, although not mandatory for assessing diagnosis. Dysfunction of lymphatic drainage often occurs neonatally. Finally, social achievement is good despite a dull average intelligence. Penetrance is incomplete and phenotypic expression is liable to substantial variability among different families as well as within a single family. Moreover, phenotype changes with ageing [Allanson et al., 1985; Sharland et al., 1993].

Nothing is known on the pathogenesis of NS, and the genes defective in this malformation syndrome are yet to be identified. However, one locus for NS was mapped recently to 12q [Jamieson et al., 1994].

In contrast, neurofibromatosis-type 1 (NF1) is a relatively easily diagnosable condition with a population frequency of ~1/3,000 and manifestations of café-au-lait spots and cutaneous or subcutaneous neurofibromata [Riccardi, 1992]. The NF1 gene was mapped to 17q11.2 and subsequently cloned. This gene spans ~350 kb of genomic DNA [Cawthon et al., 1990; Li et al., 1995] and is ubiquitously expressed. An uncommonly high mutation rate has been postulated on the ground that half the patients seem to harbor a de novo mutation. Search for mutations is hampered by the large size of the gene. Therefore, relatively few mutations have been characterized in NF1 patients to date [Upadhyaya et al., 1994].

Several other disorders have been reported in NS patients, including bleeding diathesis [Witt et al., 1988], malignant hyperthermia [Kaplan et al., 1977], trimethylaminuria [Humbert et al., 1970], and neurofibromatosis-type 1 (NF1). The latter association seems to be well established, leading some authors to discuss the existence of a new distinct entity designated as neurofibromatosis-Noonan syndrome (NF-NS) [Mendez, 1985; Opitz and Weaver, 1985; Quattrin et al., 1987;

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Abuelo and Meryash, 1988; Stern et al., 1992]. However, it has not been clearly demonstrated whether NF-NS is a unique disorder, a variant of NF1, or only the chance association of two inherited disorders. Another condition, the Watson syndrome (WS) [Watson, 1967; Allanson et al., 1991], demonstrates clinical overlap with NF1 and NS. Whether this condition is a mild form of NF-NS or a separate entity, possibly allelic to NF1, is still open.

We report on a large four-generation pedigree showing segregation of both neurofibromatosis-type 1 and Noonan syndrome. NF-NS association is discussed on the basis of clinical studies and molecular analyses using polymorphic DNA markers of the NF1 region in 17q11.2 and intragenic markers of the NF1 gene.

MATERIALS AND METHODS

Clinical Report

The index case (see Fig. 2, IV-3), a 3-month-old boy, was referred for a malformation syndrome and a cardiomyopathy. He was born to young nonconsanguineous Caucasian parents. Pregnancy was unremarkable. Fetal echography performed at 35 weeks of gestation showed enlarged biparietal and abdominal diameters (both > 90th centile), as opposed to short femur length (10th centile). Pericardial, pleural, and peritoneal effusions were noted. The four heart cavities were enlarged and a small cyst of the right kidney was reported. Vaginal delivery of a child in a state of apparent death occurred at 39 weeks of gestational age (birth weight of 2.4 kg, length of 46 cm, OFC of 36 cm). Intensive care and drainage of the pleural effusion led to rapid improvement. At age 2 months, the child developed heart failure related to a cardiomyopathy. At the time of examination, the child was short (−2.5 SD) and underweight (−2 SD). OFC was enlarged (+2 SD). He had a high, broad, and abnormally wrinkled forehead, downslanting palpebral fissures with epicanthal folds, hypertelorism, small nose with anteverted nares, midface hypoplasia, micrognathia, apparently low-set and posteriorly angulated ears, prominent anthelix with thick and detached lobules, short neck with excess of nuchal skin but without webbing, sparse and remarkably thin hair (Fig. 1). Other abnormalities included shield-chest with hypoplastic and excessively spaced nipples, short humerus and femur, short hands and fingers. Hepatosplenomegaly was also present. Genitalia were normal. Echographic examination confirmed a hypertrophic cardiomyopathy without valvular stenosis. ECG showed an electrical axis shift to the far right (>220°). A pyelic dilation of the right kidney was also found. Brain CT-scan yielded evidence of communicating hydrocephalus. Standard biological function tests failed to show any distinctive anomaly except a mild decrease in some coagulation factors. No chromosome aberration was found on lymphocyte karyotype using both prometaphase G- and R-banding techniques. All these findings were consistent with the diagnosis of Noonan syndrome (NS).

Fig. 1. Propositus at age 3 months. Noonan syndrome is evident.

Family Report

Information provided by the boy's parents indicated that NF1 segregated in the family. Due to numerous reports of clinical association between NS and NF1, we carried out a familial genetic investigation. Clinical assessment of NF1 was based on criteria as described during the NIH Consensus Development Conference in 1987 [Strumpf et al., 1987]. All affected adults had subcutaneous neurofibromata and café-au-lait spots. In some cases surgery was necessary for benign or malignant neoplastic complications. NS was ascertained on the basis of Duncan's scoring system [Duncan et al., 1981], mostly through careful clinical inspection and scrutiny of photographs at different ages. Individuals expressing no or few symptoms of NS, but having transmitted the mutation to their offspring, were designated obligate carriers. Those with only minor signs of NS without obligate carrier status were considered unaffected for purposes of linkage analysis.

Our clinical findings confirmed the coexistence of the two syndromes in the family (Table I). The propositus appeared to be affected with the most severe form of NS. The complete pedigree of the family is in Figure 2.

Phenotype Segregation Analysis

In order to test independence between NS phenotype and NF1 phenotype, the observed ratio (R_o) of "like" genotypes over "like + unlike" genotypes in each generation was calculated [Penrose, 1935]. In the dominant system characterized by both NS and NF1, genotype can be easily inferred from phenotype. If the dominant allele causing NS is *N* and the recessive wild-type allele is *n*, and if the dominant allele causing neurofibromatosis is *F* and the recessive wild-type allele is *f*, "like" genotypes are defined as [*Nn Ff*] (NS and NF1) or [*nn ff*] (neither NS nor NF1) and "unlike" genotypes are defined as [*Nn ff*] (NS without NF1) or [*nn Ff*] (NF1 without NS). Because of the lack of penetrance characterizing NS mutations, [*Nn Ff*] and [*Nn ff*] individuals may be erroneously typed [*nn Ff*] and [*nn ff*], respectively. In other words, a "like" genotype may be

typed "unlike" and an "unlike" genotype may be typed "like." The lack of penetrance of the NS mutation, therefore, diminishes the power of the test, i.e., brings observed ratio (R_o) closer to 0.5. However, it does not cause a bias such as an excess of "like" genotypes. The observed result was compared to the one calculated from the binomial distribution under the hypothesis of independence between the two phenotypic traits.

Polymorphism Typing

Blood sampling was performed with informed consent from individuals. Genomic DNA was obtained from peripheral blood leukocytes or from Epstein-Barr virus-established lymphoblastoid cell lines by classical proteinase K digestion followed by phenol-chloroform extraction.

For RFLP typing, DNA was digested with the appropriate endonucleases, electrophoresed in 0.8% agarose gels and blotted onto positively charged nylon membranes (Hybond N+, Appligene). All probes were radiolabeled by the random priming method with [α-^{32}P] dATP. Blots were treated with human DNA to compete against repeat sequences. After overnight hybridization, blots were washed and autoradiographed.

Intragenic Alu polymorphism GXAlu [Xu et al., 1991] was PCR-amplified according to the author's specifications. After polyacrylamide gel electrophoresis, blotting onto positively charged nylon membranes and hybridization with 5'end-labeled primer AluI using [γ-^{32}P] dATP, PCR products were demonstrated by autoradiography.

In addition, six markers exhibiting simple tandem repeat (STR) mapped to the long arm of chromosome 17 were analyzed after PCR amplification. These markers are either flanking or internal with respect to the NF1 gene. After amplification with fluorescein 5'-end-labeled primers, followed by addition of an internal size standard (GENESCAN 2,500-ROX, Applied Biosystems), PCR products were separated on a 6% polyacrylamide/7 M urea gel using a 373 Sequencer (Applied

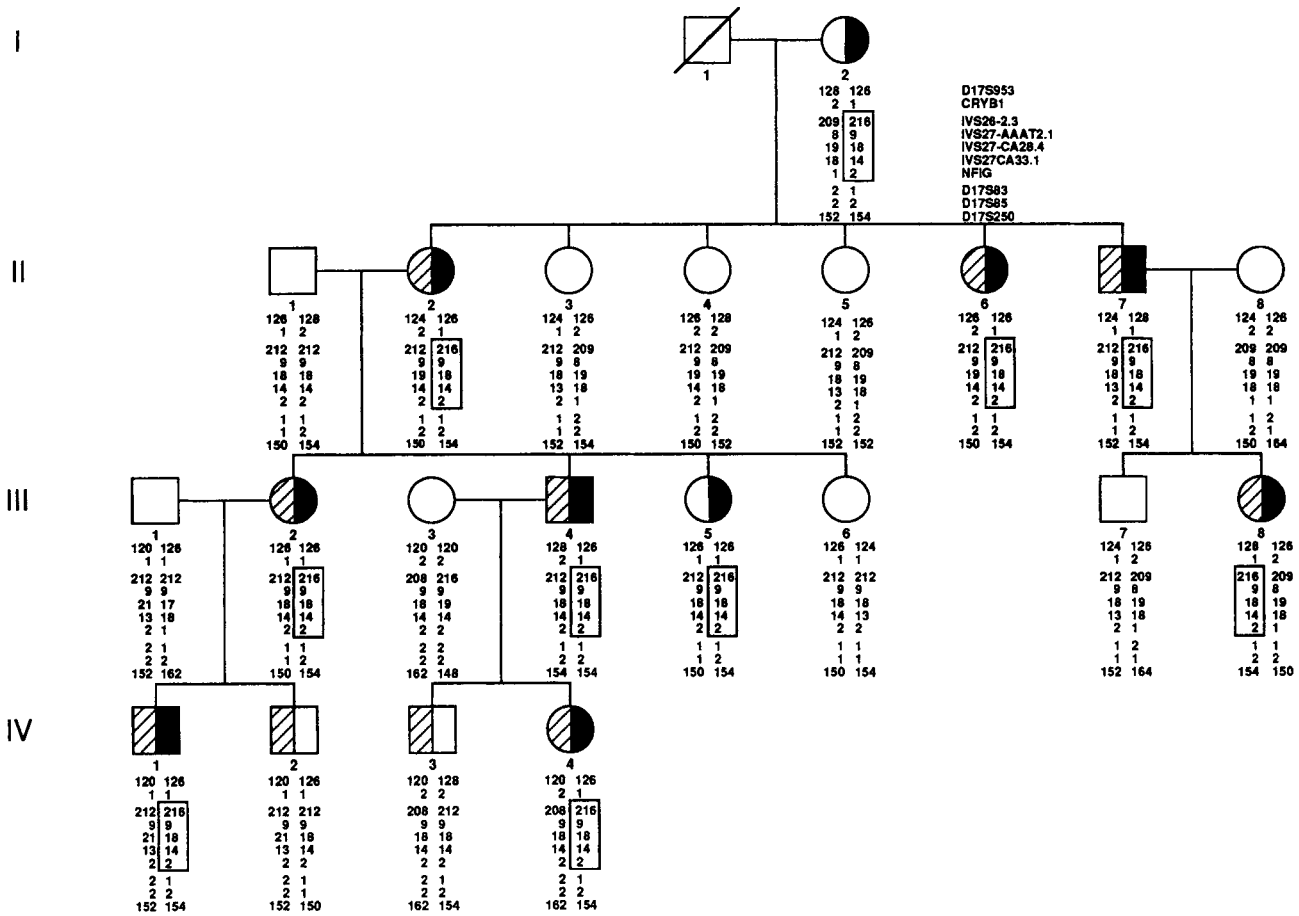

Fig. 2. Pedigree of the four-generation family with segregation of NF1 and NS. Propositus is in position IV-3. Unaffected individuals are represented with open symbols. Right blackened hemidisks or hemisquares indicate individuals affected with NF1, whereas left hatched hemidisks or hemisquares indicate individuals affected with NS or obligate carriers of the NS mutation (individuals II-2 and III-2). Status of I-1 is unknown. Haplotypes are from top to bottom alleles of loci/markers D17S953, CRYB1, IVS26-2.3, IVS27-AAAT2.1, IVS27-CA28.4, IVS27-CA33.1, NFIG, D17S83, D17S85, and D17S250, where NFIG refers to haplotypes determined by non-STR NF1 intragenic markers (Exon 30/TaqI and GXAlu). The haplotype harboring the putative NF1 mutation is boxed. Recombination occurred in individuals IV-2 and IV-3 between the NS gene and intragenic NF1 markers.

TABLE I. Clinical Findings

	Individual[a]																
	I-2	II-2	II-3	II-4	II-5	II-6	II-7	III-2	III-4	III-5	III-6	III-7	III-8	IV-1	IV-2	IV-3	IV-4
Symptom[b]																	
1	X	X				X	X		X	X			X	X			X
2	X	X				X	X	X	X	X			X				
3	X	X								X							
4		X				X	X	X	X	X			X	X	X	X	
5						X		X	X					NA	NA	NA	NA
6						X	X		X				X	X	X	X	X
7	X					X											
8		X				X	X						X	X	X	X	X
9														X	X	X	X
10						X											
11	X					X	X		X							X	
12	NA	NA				X	NA						X			X	X
13																X	
14																X	
15															X	X	
16														X			
17		X						X									
Status[c]																	
N			X	X	X												
NS		X				X	X	X	X				X	X	X	X	X
NF	X					X	X	X	X	X			X	X			X

[a]According to Figure 2.
[b](1) café-au-lait patches; (2) cutaneous/subcutaneous nodules; (3) schwannomas (benign/malignant); (4) short stature; (5) mental retardation/borderline intelligence; (6) facial anomaly (macrocephaly, course face, midface hypoplasia, downslanting palpebral fissures, low-set ears); (7) eye anomaly (squint, microphthalmy); (8) short/broad neck; (9) widely spaced nipples/thoracal anomaly (in children); (10) bone problems (cyphoscoliosis); (11) skin laxity/wrinkled; (12) cardiac anomaly (pulmonary stenosis, cardiomyopathy, significant heart murmur); (13) kidney anomaly; (14) visceromegaly; (15) lymphatic drainage anomaly; (16) hemangioma of the forehead; (17) NS obligate carrier; (NA) not assessed.
[c](N) not affected with either NF1 or NS; (NS) affected with NS or obligate carrier; (NF) affected with NF1.

Biosystems). Analysis was performed using the GENESCAN™672 (version 1.2) software (Applied Biosystems) as described in the manufacturer's manual.

A description of microsatellite and RFLP markers used in this study is given in Table II, and their respective location in 17q is shown on the genetic map of the NF1 region presented in Figure 3.

Linkage Analysis

In the linkage analysis, the NS allele was assumed to have a penetrance of 0.8 and a population frequency of 1/2,000, whereas the NF1 allele was assumed to have a penetrance of 1 and a population frequency of 1/3,000. Pairwise lod (log of the odds) scores of disease status versus marker alleles were calculated using the computer program MLINK of the LINKAGE package, version 5.1 [Lathrop et al., 1984]. Multipoint linkage analysis was performed using the LINKMAP program of the LINKAGE package. Genetic map distances between adjacent markers in the multipoint test were based on previously published linkage data (Fig. 3) [O'Connell et al., 1989; Frézal et al., 1991; Gyapay et al., 1994].

RESULTS

Phenotype Segregation Analysis

In generations III and IV, one of the parents is [*Nn Ff*], whereas the other is [*nn ff*]. Generation II is characterized by the presence of both *N* and *F* mutant alleles. Offspring can therefore result from two different mating types: either [*Nn ff*] × [*nn Ff*] (if I-1 had NS) or [*nn ff*] × [*Nn Ff*] (if the NS mutation is present but silent in I-2). In any case, as seen in generations II, III, and IV, the expected proportion of the four different resulting genotypes is 0.25, under the hypothesis of independence between the two disease loci (Table IIIA). Therefore, the expected value (R_e) is 0.5. In the family studied, the observed value (R_o) is 0.81 (Table IIIB). This figure is significantly different from the expected 0.5 value under the hypothesis of independence and the degree of significance is $P \approx 0.0104$ as calculated from the binomial distribution of (R).

Linkage Analysis for NF1

Results are summarized as haplotypes in Figure 2. As expected, the neurofibromatosis phenotype displayed in the family is thoroughly linked to intragenic markers of the NF1 gene (lod score of 4.51 at $\Theta = 0.00$). The propositus (IV-3) and his first cousin (IV-2) both received the normal NF1 allele from their affected parent.

Linkage Analysis for NS

Genotyping results are indicating that recombination occurred between the NS allele and the 17q11.2 markers. Accordingly, pairwise linkage analysis indicates that NS is not linked to the NF1 locus in 17q11.2 in this family (Table IV). As shown in Figure 4, multipoint linkage analysis demonstrates that NS is excluded from a region spanning 15 cM and 11 cM centromeric and telomeric respectively, to the NF1 locus.

DISCUSSION

In the past few years, numerous observations relating the simultaneous occurrence of NS with NF1 lead some authors to postulate the existence of a distinct clinical entity referred to as neurofibromatosis-Noonan syndrome (NF-NS). The relevance and possible molecular basis of such an entity are still debated. However, several hypotheses can be formulated to explain the pathogenesis of this association.

The first hypothesis states that NF-NS represents the likely concomitance of two otherwise distinct mutations in the same individual, since both conditions are frequent autosomal dominant disorders. In at least one observation, it has been demonstrated that one individual was affected with NF1 and NS as the result of two independently inherited mutations [Colley et al., 1989]. However, phenotype segregation analysis in the large four-generation pedigree presented here indicates preferential segregation of NS with NF1 ($P \approx$

TABLE II. Polymorphic Markers

A. Microsatellite loci typed

Microsatellite marker name	Fluorescently labeled forward primer	Unlabeled reverse primer
AFM304xh5	ACTATCCGCCCAATACA	AAGGGCTTGCTTTGAC
NF 1 Gene		
IVS26-2.3	AGGCCAGGAGTTCAAGACCA	ATGAGCCACTGTGCCCAATC
IVS27-AAAT2.1	GAGCCAAGATCGCACCACTG	CTTTGGTGGGATACTATTACGTTG
IVS27-CA28.4	TGAAGTATGCAGTTTTCCAG	GGCTAAGTGTAAACGCAAAG
IVS27-CA33.1	TAGATTATATGGGACAGAAAATG	CTTGAGGTGATGACAGGATG
mfd15	GGAAGAATCAAATAGACAAT	GCTGGCCATATATATATTTAAACC

B. RFLP loci typed

Probe name	Enzyme	Fragment sizes (kb)
pB8.2	MspI	2.4/1.7
NF 1 Exon 30	TaqI	7.0/6.5
p11-2C11.5	TaqI	2.0/1.8
p11-2C11.7	BamHI	4.5/4.4
p11-2F9.8	TaqI	12.0/6.5

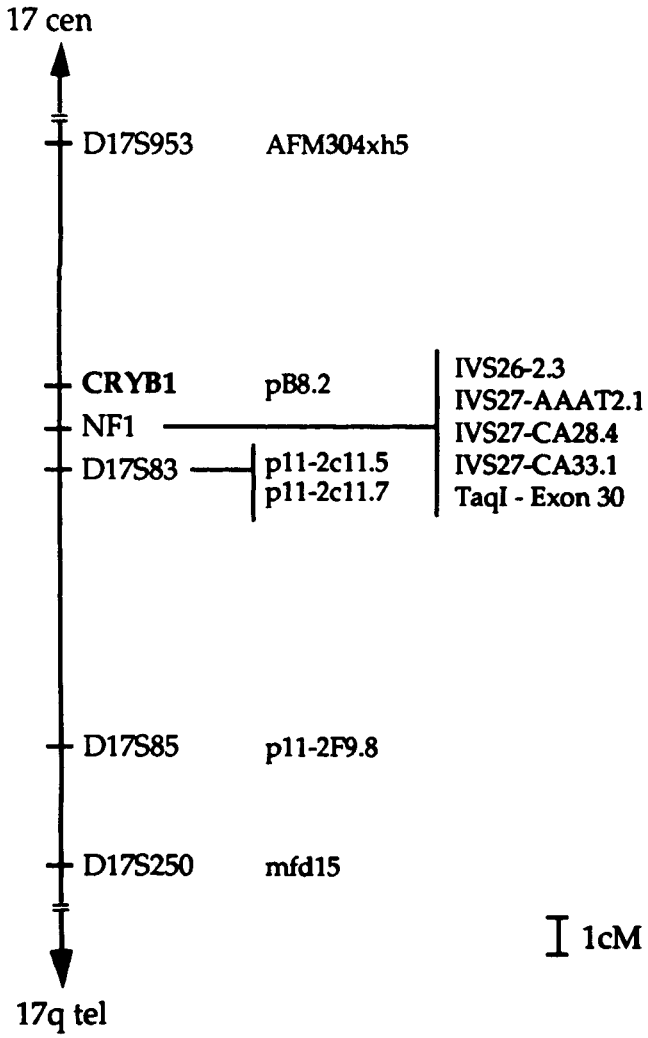

Fig. 3. Genetic map of chromosome 17 around the NF1 locus. The genetic distance between two adjacent loci is proportionate to the length of the interval on the draft. The scale indicates 1 centiMorgan (cM).

0.0104), suggesting linkage between the two conditions. One important aspect of this result is that the propositus (Fig. 2, IV-3) has NS and is devoid of symptoms suggesting NF1, which rules out an ascertainment bias. In other words, it can be postulated that in this family the NF-NS association is not the result of chance occurrence of two independently inherited mutations. The NS allele segregating in this family was also excluded from the previously described Noonan locus in 12q (data not shown).

A second hypothesis states that NF-NS is an allelic variant of NF1. Indeed, the phenotypic spectrum of NF1 sometimes includes minor anomalies and malformations such as those seen in NS. Upadhyaya et al. [1992] reported a 80 kilobase-pair deletion of the NF1 gene in a patient with WS; Tassabehji et al. [1993] described a 42 base-pair tandem duplication in exon 28 of the NF1 gene in a family with WS and NS. These reported mutations are confined to the NF1 gene and if the patients can be typed NF-NS or WS, are consistent with the hypothesis of NF-NS or WS being allelic to NF1.

If NF-NS is an allelic variant of NF1, the first alternative is that the NF1 mutation has pleiotropic effects, i.e., impairment of neurofibromin can result in nonspecific Noonan-like phenotype. Neurofibromin is present in a wide range of tissues during embryogenesis and adulthood [Maneri Daston and Ratner, 1992; Huynh et al., 1994]. Consistent data have been collected arguing that this protein is crucial for the normal development of numerous tissues and especially the cardiac muscle [Gutmann et al., 1993; Brannan et al., 1994]. Impairment of heart development is a major clinical aspect in NS. Mutations of the NF1 gene could therefore be responsible for NF1 together with a series of malformations, including congenital heart disease, thereby accounting for the clinical overlap between NF1 and NS.

The second alternative is that disruption of sequences lying within or near the neurofibromin gene accounts for the associated syndrome, i.e., could per se induce a bona fide Noonan syndrome. Indeed, mutations observed in NF-NS or WS patients do not rule out this alternative or even a possible second mutation affecting a distinct gene at the same locus. Fine genetic regulation is probably required for transcriptional balance between the neurofibromin gene and the three others genes (OMGP, EVI2A, EVI2B) transcribed in the opposite direction, from the opposite DNA strand. Disruption of cis-acting elements or direct mutation of these three and other possible neighboring genes could explain an associated syndrome. More recently, various large deletions of the NF1 gene were characterized in patients with neurofibromatosis who were otherwise affected with mental retardation and minor facial anomalies [Kayes et al., 1994]. These deletions remove the entire NF1 gene sequence and are therefore consistent with a possible contiguous gene syndrome.

In the family studied, linkage analysis using DNA polymorphisms demonstrates that the peripheral neurofibromatosis phenotype segregates with intragenic NF1 markers ($Z_{max} = 4.51$; $\Theta_{max} = 0.00$), indicating that the causative mutation lies within the NF1 locus. As described in the case report, the propositus (Fig. 2, IV-3) has full-blown NS. His first cousin (Fig. 2, IV-2) has a milder form of the same condition. Neither of them have clinical findings of NF1. Conversely, both received the normal NF1 allele from their affected parent. Therefore, it can be postulated that a recombination event occurred between the NF1 and NS loci in these two individuals. Hence, the NS allele is not allelic to NF1 and the NS gene is not imbedded in the NF1 gene sequence. Such results are also in agreement with the absence of a major rearrangement of the neurofibromin gene in this family.

In the Family Studied, NF-NS Phenotype Results From Two Genetically Linked But Distinct Mutations

Cosegregation of distinct but genetically linked mutations in a family can mimic a pseudo-contiguous gene syndrome. Such a mechanism has been illustrated in a

TABLE III. Segregation Analysis

A. Expected gamete and offspring proportions[a]

[*nn ff*] × [*Nn Ff*] mating				
gametes	*NF*	*Nf*	*nF*	*nf*
	0.25	0.25	0.25	0.25
nf	[*Nn Ff*]	[*Nn ff*]	[*nn Ff*]	[*nn ff*]
1	0.25	0.25	0.25	0.25
[*Nn ff*] × [*nn Ff*] mating				
gametes	*nF*	*nf*		
	0.5	0.5		
Nf	[*Nn Ff*]	[*Nn ff*]		
0.5	0.25	0.25		
nf	[*nn Ff*]	[*nn ff*]		
0.5	0.25	0.25		

B. Observed offspring proportions[b]

Genotypes	Like		Unlike		Total
	[*Nn Ff*]	[*nn ff*]	[*Nn ff*]	[*nn Ff*]	
Generation II	3	3	0	0	6
Generation III	3	2	0	1	6
Generation IV	2	0	2	0	4
Total	8	5	2	1	16
	13		3		

[a]A indicates expected gamete and offspring proportions resulting from the two different possible mating types. In whichever case, the expected (R) value (R_e) is:

$$R_e = \frac{[Nn\,Ff] + [nn\,ff]}{([Nn\,Ff] + [nn\,ff]) + ([Nn\,ff] + [nn\,Ff])}, \text{or}$$

$$R_e = \frac{0.25 + 0.25}{(0.25 + 0.25) + (0.25 + 0.25)}, \text{or}$$

$$R_e = 0.5.$$

[b]B indicates different genotypes observed in each generation. Of 16 individuals, 13 have a "like" genotype and 3 have an "unlike" one. The observed (R) value is 13/16. The chance that this figure be equal or greater than 13/16 is:

$$P = P(R_o \geq \frac{13}{16}) = \sum_{n=13}^{16} n \frac{(0.5)^{16} \times 16!}{n! \times (16 - n)!}$$

with $P \approx 0.0104$.
Therefore, (R_o) is significantly different from (R_e) and P gives the degree of significance.

large family where a genetic predisposition for breast and ovarian cancer segregates with palmoplantar keratoderma [Torchard et al., 1994]. A point mutation of cytokeratin K9 gene in 17q21 was suggested to be responsible for palmoplantar keratoderma. This mutation was the unlikely cause of the observed malignancies that were linked to a putative mutation of the BRCA1 gene. In this observation, the mutated genes were so close that no recombination occurred out of the 24 studied meioses.

In the pedigree presented here, statistically significant cosegregation of the NF1 and NS phenotypes together with observation of two recombination events out of 16 meioses indicate that the NF-NS phenotype is caused by two genetically linked but distinct mutations. These data strongly support the hypothesis for a gene mutated in NS being on chromosome 17, near the NF1 locus. Linkage analysis performed between NS and the markers studied excludes the NS allele from a region spanning 26 cM over and around the NF1 locus.

TABLE IV. Pairwise Linkage Analysis for Noonan Syndrome*

	Recombination fraction (Θ)								
	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Zmax	Θmax
Locus									
D17S953	−6.66	−2.91	−1.43	−0.77	−0.20	−0.01	0.02	0.02	0.632
CRYB1	−2.57	−0.88	−0.26	−0.05	0.07	0.06	0.02	0.08	0.765
NF1 gene	−1.48	−0.87	0.26	0.64	0.74	0.51	0.16	0.76	0.171
D17S83	0.99	0.97	0.88	0.76	0.52	0.28	0.08	0.99	0.000
D17S85	1.05	1.02	0.92	0.80	0.53	0.27	0.07	**1.05**	**0.000**
D17S250	−7.8	−3.61	−2.09	−1.37	−0.58	−0.20	−0.04	0.00	0.5

*Maximum lod score was observed for locus D17585 (boldface).

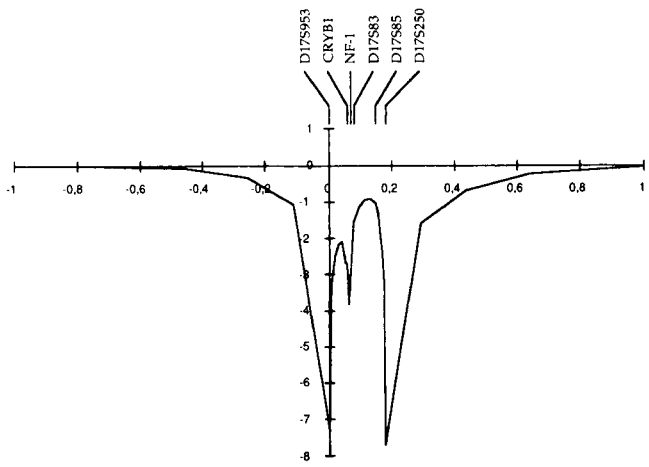

Fig. 4. Multipoint linkage map for NS. Abscissa indicates genetic distances in Morgans, where position 0 was arbitrarily chosen for locus D17S953. Ordinate represents lod scores.

However, we cannot rule out an unlikely but possible compound phenotype with a specific mutation of the NF1 gene leading to NF-NS with incomplete penetrance for NS and a distinct mutation leading to bona fide NS.

Mutations of distinct genes in neighboring loci such as described here could account for some of the NF-NS cases in the general population. Indeed, the fact that these two genes appear in linkage disequilibrium is consistent with familial cosegregation of two disease alleles, whatever the underlying molecular mechanism. However, if the occurrence of two independent mutations in the same individual is the product of each single mutation rate (1/3,000 for NF1 and 1/2,000 for NS), sporadic cases with NF-NS due to this two-hit mechanism should be extremely rare. Allelism to NF1 is probably what accounts for most NF-NS or WS cases in the general population, whatever precise mechanism leads to impaired development in these patients. It is striking, however, that a large number of individuals diagnosed with NF-NS or WS do not display full-blown NS and would probably not be diagnosed NS, were they not affected with NF1.

Noonan Syndrome Is a Heterogeneous Condition

Clinical heterogeneity of NS is exemplified by the multiple associated disorders seen in NS patients and is probably the result of genetic heterogeneity. Efforts aimed at mapping a gene responsible for NS was recently successful, assigning one of the loci to 12q. However, some families do not map to this locus [Jamieson et al., 1994].

Our results strongly suggest the existence of a new NS locus on chromosome 17. Localization to chromosome 17 has been frequently hypothesized because of the clinical association of NS with NF1. However, mapping efforts only demonstrated linkage exclusion [Sharland et al., 1992, Flintoff et al., 1993, Edman Ahlbom et al., 1995]. Other loci also have been candidates for harboring a gene mutated in NS but without conclusion to date. Whether such genes belong to a superfamily with sequence homologies or are implied in a common metabolic pathway is still open to question.

Linkage analysis on large and genetically homogeneous pedigrees together with the molecular characterization of selected chromosomal rearrangements will be a prerequisite for the mapping and cloning of genes mutated in NS. Identification of these genes will help us to better understand such an enigmatic malformation condition in humans.

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